THE CLOSING RESPONSE IN DIONAEA[1]

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(WITH ONE FIGURE)

Despite the attention attracted during the past century by the extraordinary reactions of *Dionaea muscipula* Ellis, there remains much uncertainty with regard to many points in its behavior. This uncertainty has led the present writers to carry out the work here reported.

The structure of the leaf is so well known that its description here is unnecessary. It may be well, however, to recall the fact that the leaf blade consists of two valves, each of which bears upon its upper surface three short, rather stiff bristles. Mechanical contact with these bristles causes the two valves to close together upon each other.

Relation of intensity to number of stimuli

DARWIN (3), SACHS (9), BATALIN (1), DETMER (4), MUNK (7), and others held that one contact stimulus was sufficient to produce the closing response. DARWIN (3) and BURDON-SANDERSON (2) showed that an extremely slight stimulus might be inadequate, while MACFARLANE (6) concluded from his researches that under ordinary conditions two mechanical stimuli are always necessary. In investigating this question it is of course necessary to avoid all possibility of shaking, which might cause uncertainty as to the number of stimuli applied. In the present experiments a stiff straw was so supported that it could be adjusted to any desired position and moved mechanically by means of a rack and pinion and milled head. By this method the movement of the straw could be accurately controlled, and the number of stimuli definitely known.

Leaves of plants which had been kept at 15° C. for one to two hours were stimulated by pressure upon one of the sensitive hairs, and it was found that closure almost never resulted after one such stimulus, even though the hair was bent down so as to touch the

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leaf. If, however, a second stimulus was applied either on the same or another hair, after an interval of 1.5 to 20 seconds the leaf responded, and in nearly every case by complete closure. The leaves of plants which had been kept about the same length of time at 35° C. frequently responded to one contact stimulus, while those kept at 40° C. closed with the first stimulus about as often as with the second. Slight individual differences were sometimes found in the leaves tested. These results seem to show that while under ordinary conditions two mechanical stimuli are usually necessary for closure, the number is not fixed, but varies with the environment and to some extent with different leaves.

The question now arises as to whether a certain amount of stimulation, rather than a certain number of stimuli, is required to effect closure. This was first investigated by the use of electrical stimuli, the intensity of which could be accurately controlled. MACFARLANE (6) was inclined toward the belief that two electrical shocks are necessary to cause the closing response.

Two series of experiments were carried out, in one of which the terminals of an induction coil were connected by wires with the petiole and keel, and in the other with the petiole and a sensitive hair. These connections were made by one gentle touch in such a manner as not to cause closure as a result of contact. The leaves were allowed five minutes in which to recover from any after-effect of the contact stimulus. They were then stimulated with opening shocks at intervals of 15 seconds, a single dry cell being used in the primary circuit. The result in both series was essentially the same, the number of shocks necessary to cause closure increasing as the intensity of the stimulus decreased. One strong shock was always sufficient to cause the response, but when weaker shocks were applied the number increased until in one case 26 were required. As with mechanical stimuli, the leaves showed slight individual variations. These results are shown in table I, in which the numbers indicating the position of the sliding secondary coil indicate the intensity of the induced current produced, the greatest electromotive force corresponding, of course, to the position marked 0, while the lowest corresponds to position 6. The effect of a stronger current, obtained by the use of two cells in place of one in the primary circuit, was

tested at position o, but no difference was observable between the response in this case and that in which only a single cell was used. Partial closures were obtained before the complete closures in four cases with the first arrangement of contacts, and in a single case with the second arrangement. These are indicated by footnotes below the table.

TABLE I

NUMBER OF ELECTRIC SHOCKS REQUIRED TO PRODUCE COMPLETE CLOSURE WITH VARYING INTENSITY OF CURRENT; SHOCKS AT INTERVALS OF 15 SECONDS

CONTACT ON SENSITIVE HAIR AND PETIOLE								
Position of coil	No. of test	0	½	2	3	4	5	6
Number of shocks required for complete closure	1	1	1	1	4*	6‡	7	10¶
	2		2	2	3‡	1†		26
	3		1					
Average		1.0	1.3	1.5	3.5	3.5	7.0	18.0

CONTACTS ON KEEL AND PETIOLE							
Position of coil	No. of test	0	½	2	3	4	5
Number of shocks required for complete closure	1	1	1	2	1	3†	4
	2		1	2	2	2	5
	3		1				
Average		1.0	1.0	2.0	1.5	2.5	4.5

* Partial closure with 3 shocks.
† Partial closure with 2 shocks.
‡ Partial closure with 5 shocks.
¶ Partial closure with 9 shocks.

It is apparent from these tests that closure is due to intensity of stimulation rather than to number of stimuli, and that there exists a definite after-effect with a duration of over 15 seconds, the summation of these after-effects finally producing the response in the case of the weaker electric shocks.

We have heretofore considered mechanical stimuli as though all were of the same intensity, a condition which appears to be true if the hair is markedly bent. As has been stated, leaves kept at a temperature of 15° C. usually close on the application of the second stimulus, and in this case it appears to make no difference whether the bending of the sensitive hair is comparatively slight, or great enough to bring the hair against the leaf. At higher temperatures the leaf responds to one stimulus as frequently with slight as with more marked bending of the sensitive hairs. This is shown even more conclusively by the fact that at a temperature of 15° C. the leaf fails to respond when the hair is bent down against the leaf by one movement, while less bending, if accomplished by two movements, brings about response.

If, however, the bending is so slight as to be scarcely apparent, three to five stimuli, rather than two, are usually necessary to produce response. It appears, in general, that in both mechanical and electrical stimulation closure is due to the amount of stimulation rather than to the number of stimuli, but the amount of stimulation appears not always to be proportional to the amount of bending of the sensitive hair. If this is true, the number of contact stimuli necessary to cause closure should increase with the length of the time

TABLE II

RESPONSE TO CONSECUTIVE CONTACT STIMULI AT VARYING TIME INTERVALS

TIME INTERVAL BETWEEN STIMULI	NO. OF EXPERIMENT	NUMBER OF STIMULI								
		1	2	3	4	5	6	7	8	9
	1	—	+							
20 seconds	2	—	+							
	3	—	+							
	4	—	+							
	1	—	P	+						
1 minute	2	—	P	+						
	3	—	—	P	+					
	4	—	—	—	P	+				
	1	—	—	—	P	+				
	2	—	—	—	—	P	+			
2 minutes	3	—	—	—	—	P	+			
	4	—	—	—	—	P	+			
	5	—	—	—	—	—	P	+		
	6	—	—	—	—	—	P	+		
	1	—	—	—	—	—	P	P	+	
3 minutes	2	—	—	—	—	—	—	P	P	+
	3	—	—	—	—	—	—	—	P	+

interval between them, since the effect of stimulation would be expected to disappear to a greater extent as the length of the time interval is increased. This hypothesis was tested with the result that leaves at a temperature of 15° C., stimulated mechanically at intervals of 20 seconds, closed with the second stimulus; when the interval was increased to a minute, they responded with 2 to 5 stimuli; at intervals of 2 minutes, a response was produced with 5 to 7 stimuli; while at intervals of 3 minutes, about 6 to 9 stimuli were necessary to bring about closure. These results are shown in table II, in which — denotes no response; P, partial closure; +, complete closure.

It is seen at once that with increasing length of time interval the number of stimuli required to produce a response increases also. Considering only the number of stimuli required for complete closure, these results are tabulated in table III.

TABLE III

NUMBER OF STIMULI NECESSARY TO PRODUCE COMPLETE CLOSURE, THE TIME INTERVAL VARYING FROM 20 SECONDS TO 3 MINUTES

Experiment no.	Time intervals between stimuli			
	20 seconds	1 minute	2 minutes	3 minutes
1	2	3	5	8
2	2	3	6	9
3	2	4	6	9
4	2	5	6	
5			7	
6			7	
Average	2.0	3.8	6.2	8.7

The averages from this table are plotted on the graph of *fig. 1*, in which the abcissas represent the length of the time intervals between stimuli, and the ordinates the average number of stimuli required for complete closure. In this region the graph shows only a slight curvature. It is nearly a straight line, which would seem to denote that at intensities ranging from 20 seconds to 3 minutes—and of course for these particular plants—the number of stimuli necessary for complete response varies almost directly with the length of the intervals.

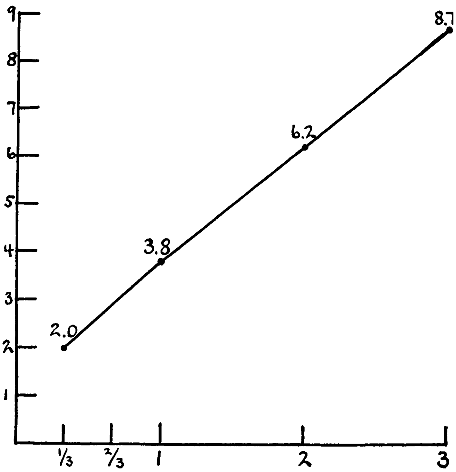

Fig. 1

It may be concluded from the foregoing that leaves seem to respond by closing to a definite amount of accumulated effect, possibly the

production of some chemical substance as the result of excitation; that the amount of this effect varies with the intensity of the stimulus; and that the amount of stimulation necessary for closure varies with different leaves, probably with different conditions of the plant.

Localization of perception

DARWIN (3) held that the surface of the blade is very slightly, and the footstalk not at all sensitive. MACFARLANE (6) showed that scraping the surface or squeezing the blade with steel forceps would cause closure, and concluded that "the leaf of Dionaea, then, is truly sensitive throughout its halves to mechanical stimulation, but the capacity for receiving sensation impulses is highly concentrated in the hairs." This writer states, however, that two pinches with the forceps are required to cause closure. In our experiments, leaves responded to one, two, or even more such pinches, according to the strength of the stimulus and the condition of the leaf. It thus appears that MACFARLANE'S conclusion is supported, in that all parts of the leaf are sensitive, and the question is raised whether the protoplasm of all parts may not be equally sensitive to stimulation, and that the hair functions simply as a mechanism which compresses certain cells. OUDEMANS (8) and BATALIN (1) came to the conclusion that the base of the hair is the sensitive region, while HABERLANDT (5) has pointed out near the base of the hair a layer of cells which appear especially fitted for being bent, and thus having their contents compressed. The latter author considered this layer the sensitive region, and expressed surprise that the rest of the leaf should be sensitive at all.

When a hair is stimulated by contact, it is first bent laterally and downward, held in this deformed position for a short time, and then allowed to spring back to its original position. If stimulation be due to a compression of cells at the base of the hair, the downward movement alone should cause closure. To test this, a stiff straw was supported as in former experiments, so that it could be accurately adjusted and held definitely in any position. Two downward thrusts on the same sensitive hair without an intervening release caused in every case at 15° complete closure. With a temperature

of 35° C. to 40° C. the leaves frequently responded to one downward thrust without a release.

When a sensitive hair was bent down and held mechanically in that position for 5 minutes, the leaf nearly always failed to respond. This experiment was repeated many times, but response followed in only a very few cases, and then only a few seconds after the contact. These exceptional closures seem to have been due to the downward thrust.

In order to determine the effect of the upward movement of the hair, the latter was bent down carefully against the leaf without causing closure, and held in that position for a period of 5 minutes in order that the effect of the compression stimulus might disappear. The hair was then allowed to spring back to its original position by two successive upward movements. This experiment was repeated many times, but the leaves never exhibited any response. The same operation was then repeated with the addition of alternate bendings and releases subsequent to the two upward movements, at intervals of 15 seconds. In no case did the leaf respond to a release, but always to a downward movement. The leaf is thus seen to respond only to a downward bending of the hair, and it seems probable that stimulation is due to a compression of the cells at its base, and that this compression is analogous to the compression of the cells of the blade when the latter is squeezed with forceps.

That the bending of the cells at the base is alone sufficient to cause response was shown in the following manner. A hair was cut off near the base and the leaf allowed ample time to recover from any possible shock produced by the cutting. The remaining portion was then pressed with a needle. In this operation it could be seen by means of a lens that bending took place only in the region where lie the cells shown by HABERLANDT to be especially suited to a compression stimulus. In such cases the leaves responded normally. That simple bending of the sensitive hair, without contact with a hard object, is sufficient to cause closure was shown by directing jets of air against it, when the leaf responded as if stimulated by contact.

We have seen that all parts of the leaf are sensitive to mechanical pressure, and that the hair probably functions as a mechanism for the compression of certain cells. It next remains to be seen whether

the protoplasm of these cells is more sensitive than that of the other cells of the leaf. Inspection of table I will show that when electrical shock was conducted through the keel to the petiole, and therefore not passed through the region at the base of the hair, it was just as effective, or perhaps slightly more so, than when passed through the sensitive hair itself.

It was found that water at room temperature when carefully applied to either surface of the leaf blade does not cause it to close. Water at 65° C., however, will cause closure without harming the leaf, and this response is just as marked, so far as could be determined, when the water is applied to the back of the leaf as when applied to the inner surface or to the bases of the sensitive hairs. These experiments with heat and electricity seem to indicate that the other cells of the blade are just as sensitive as those at the bases of the hairs. Contrary to DARWIN's opinion, the petiole is also sensitive, as we have found that passage of electrical shocks through it will cause closure of the valves. In this case, however, a much stronger stimulation is required to effect closure than when the shock is applied directly to the blade. This difference may very well be due to loss in conduction of the stimulus from the petiole to the region of bending.

Stimuli of various forms

In the experiments already recorded, it has been shown that closure may result from the application of mechanical, electrical, and thermal stimuli. DARWIN (3), MACFARLANE (6), and others have shown that the leaf may also be closed as the result of chemical stimulation. For convenience, these various kinds of stimuli may be discussed separately.

MECHANICAL STIMULI.—It has been seen that all parts of the leaf are sensitive to mechanical stimulation, and that this acts through compression of cells. DARWIN (3) states that water falling on the sensitive hairs does not cause closure, while MACFARLANE (6) observed that a steady stream directed against a hair, or the gradual immersion of the leaf, does cause the response. In our experiments it was found that the leaves could be filled carefully with water, or wholly immersed, without causing the response, but that the dropping of water on the hairs, or the movement of the leaf while immersed, caused it to close

if the hairs were thereby bent. The effect of water seems to be entirely due, therefore, to its mechanical action.

It is well known that a slight shaking will cause movement in the leaf of Mimosa. Experiments were undertaken to determine whether the leaf of Dionaea would also respond to shaking. These were not conclusive, but showed at least that the leaves can endure much shaking without showing response. In one case a long-petioled leaf was arranged so that it was struck near the distal end on alternate sides by the bar of a metronome beating 200 times per minute; and after 45 minutes the leaf showed no change. When the leaves of Mimosa are shaken, the large leaflets offer a considerable resistance to the air, which, together with their inertia, results in the bending of them and of the pulvini. The only parts of a Dionaea leaf which could be so affected are the cells at the bases of the sensitive hairs, as all the rest of the leaf is comparatively rigid. The hairs are so slender that they offer little resistance to the air, and on account of their small mass have a relatively slight inertia, and so when moved through the air can have little tendency to bend the cells at their bases. In experiments, however, in which the leaves were shaken under water, which offers a greater resistance to the passage of the hairs, the result was the same as in a Mimosa leaf shaken in air. It would thus seem that the nature of the response is the same in the two cases, the effect of the denser medium in the former balancing the effect of the large leaflets in the latter. This conclusion is further supported by the experiment already noted, in which the leaf closed as a result of bending produced in a sensitive hair by jets of air directed against it.

MACFARLANE (6) found that leaves stimulated twice mechanically at an interval of 0.25 second did not close on the second stimulus. If, however, the interval was 0.33 second or more they did close on the second stimulus.

Leaves which had been kept for an hour at 15° C. were stimulated twice mechanically at intervals of 0.25 to 2 seconds. When the interval was less than 0.75 second, response never followed; when it was 1 second, response was frequent; while at intervals of 1.5 seconds or more the leaves invariably closed. At temperatures of 35° C. to 40° C. the leaves rarely failed to respond to two mechanical stimuli separated by an interval of 0.25 second. They always

responded when the interval was longer than this and failed to respond with shorter intervals. This is added evidence that the leaves respond more readily at higher temperatures than at lower. As in the case of animal muscle, there seems to be in Dionaea a short interval after one stimulus during which another has no effect.

THERMAL STIMULI.—MACFARLANE (6) allowed drops of water at temperatures varying from 50° C. to 75° C. to fall upon open leaves. At the higher temperature one drop caused closure, while at the lower several applications were necessary. Only four of the leaves so treated reopened, and he says, "the subsequent fate of most of the leaves points to a permanent injury." It is not stated whether or not the water was dropped on the sensitive hairs. The present writers found that water at room temperature, dropped directly upon the hairs, causes closure, while the gentle application of water at 50° C. does not cause the response. When warm water is dropped in this manner, it is of course cooled somewhat before it reaches the leaf. Since it is possible to interpret the cases reported by MACFARLANE as being due either to a heat stimulus, to a mechanical stimulation of the sensitive hairs, or to injury, it was thought advisable to reinvestigate the effects of heat. Water at 65° C. was applied in some cases to the inner, and in other cases to the outer surface of the leaves. In all cases closure resulted. These leaves exhibited no appearance of injury, and after reopening responded again quite normally. As a control for these experiments, water at room temperature was applied for several minutes to both inner and outer surfaces of the leaves without effect. This seems to indicate that heat causes the closing response. Water at 75° C. was then applied as above. Closure resulted in all cases, but two days later the parts of the leaves touched by the water were dead and black. Only small areas on some of them had been injured, and in several such cases the rest of the leaf responded normally. We can also confirm MACFARLANE'S statement that cold water will cause closure. This is true whether it be applied to the inner or to the outer surface.

ELECTRICAL STIMULI.—Experiments already described show that electrical stimuli, when applied to various parts of the leaf and petiole, cause closure, and that the effect depends upon the intensity of the stimulation rather than upon the number of stimuli. The response

is brought about by either opening or closing shocks from an induction coil, but the former are much more effective than the latter. The feeble effect of closing shocks is shown by the fact that when as many as 100 of these were applied to leaves at intervals of 15 seconds, the coil being at position 0 (see table I), the leaves showed no sign of response. When the closing shocks were followed by opening shocks, the leaves responded to two of the latter as if nothing had preceded them. In another case two dry cells were used in the primary circuit, and the leaf closed on the 21st closing shock. Continuous current from a dry cell failed to cause closure.

EFFECT OF COMBINING STIMULI OF TWO FORMS.—As has been shown, leaves kept at 15° C. usually respond to two marked bendings of the sensitive hairs. A series of such plants was stimulated first by a single contact and then by an electric shock of such strength that two of these would be required to cause closure if used alone. These plants usually responded on the first electrical stimulus, while in two control series, in one of which only contact stimuli were used and in the other only electrical stimuli, they usually responded on the second stimulus. Table IV represents these three series.

TABLE IV

STIMULATION OF SENSITIVE HAIR BY MECHANICAL AND ELECTRICAL STIMULI

SERIES I HAIR STIMULATED BY ONE MECHANICAL CONTACT FOLLOWED BY OPENING SHOCKS				SERIES II HAIR STIMULATED ONLY BY OPENING SHOCKS OF THE SAME INTENSITY AS THOSE OF SERIES I		SERIES III HAIR STIMULATED ONLY BY MECHANICAL CONTACTS SIMILAR TO THOSE OF SERIES I	
Experiment number	Number of shocks required for complete closure			Experiment number	No. of shocks required for complete closure	Experiment number	No. of contacts required for complete closure
	Mechanical	Electrical	Total				
1.........	1	1	2	1	2	1	2
2.........	1	1	2	2	2	2	2
3.........	1	1	2	3	3	3	2
4.........	1	2	3	4	2	4	2
5.........	1	1	2	5	2	5	2

The results given in table IV seem to show that stimuli of different forms produce a similar internal effect, and that the leaves respond, as has already been pointed out, only with the accumulation of a certain amount of this effect.

Summary

The closing response in Dionaea depends upon the intensity rather than upon the number of stimuli, the number of stimuli required varying in the inverse order of their intensity.

Response is normally brought about by the compression of certain cells at the bases of the sensitive hairs, but the compression of other cells of the blade also causes closure, and it is probable that the latter cells are equally sensitive with the cells at the bases of the hairs, as is indicated by electrical and thermal stimulation.

The closing response follows the application of mechanical, electrical, and thermal stimulation. It also follows a combination of stimuli of two kinds when consecutively applied, the individual stimuli being of an intensity such that either alone would be insufficient.

The effect of mechanical stimulation is due to compression of cells, and not to contact with a hard object, continued pressure, or release of pressure. The failure of the leaf to respond to shaking is probably connected with the small inertia of the sensitive hairs, and the slight resistance offered by the air to their passage through it.

Water at room temperature causes closure only when it bends a sensitive hair.

After one mechanical stimulus there is a short period during which a second mechanical stimulus is ineffective.

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